Nisin affects the growth of *Listeria monocytogenes* on ready-to-eat turkey ham stored at four degrees Celsius for sixty-three days

A. Ruiz,* S. K. Williams,*[1] N. Djeri,* A. Hinton Jr.,† and G. E. Rodrick‡

*Department of Animal Sciences, PO Box 110910, University of Florida, Gainesville 32611-0910; †USDA-Agricultural Research Service, Richard B. Russell Agricultural Research Center, Athens, GA 30605; and ‡Food Science and Human Nutrition Department, University of Florida, Gainesville 32601

ABSTRACT The objectives of this study were to determine pH, anti-*Listeria* and general antimicrobial properties of nisin on ready-to-eat vacuum-packaged diced turkey ham inoculated with *Listeria monocytogenes*, and the usage level that would exert maximum antimicrobial effect during 63 d of storage. Ready-to-eat diced turkey ham was inoculated with a 5-strain *L. monocytogenes* cocktail; treated with 0.2, 0.3, 0.4, and 0.5% nisin treatment solutions; vacuum-packaged; stored at 4 ± 1°C for 63 d; and analyzed at 1-wk intervals for total aerobic counts, pH, *L. monocytogenes*, and lactic acid bacteria. Antimicrobial effectiveness of nisin increased as concentration increased from 0.2 to 0.5%. Aerobic plate counts for 0.4 and 0.5% nisin were lower ($P < 0.05$) than negative and positive controls. All nisin treatments resulted in 4 log reductions ($P < 0.05$) in *L. monocytogenes* when compared with the positive control on d 0. Four log reductions were also observed on d 7 for 0.4% nisin treatment and d 7 and 14 for 0.5% nisin treatment when compared with the positive control. *Listeria monocytogenes* counts decreased from 4.97 log cfu/g on d 0 and remained less than 2 log cfu/g through 63 d of storage for the 0.5% nisin treatment. Lactic acid bacteria counts were lower ($P < 0.05$) for 0.5% nisin treatment when compared with positive and negative controls from 28 through 63 d. Except for d 56 and 63, pH was similar ($P < 0.05$) for all treatments. This study revealed that nisin could be used for postprocessing intervention to control *L. monocytogenes* in ready-to-eat poultry products.

Key words: nisin, bacteriocin, turkey ham, ready-to-eat product, *Listeria monocytogenes*

2010 Poultry Science 89:353–358
doi:10.3382/ps.2008-00503

INTRODUCTION

Listeria monocytogenes is the causative agent of listeriosis and has resulted in numerous major foodborne outbreaks worldwide (Gombas et al., 2003). The ability of *L. monocytogenes* to grow at temperatures ranging from 0 to 45°C (Barbosa et al., 1994), tolerate salt (Farber and Peterkin, 1991), and grow at a relatively low pH (Bell and Kyriakides, 2005) causes the bacteria to be difficult to control in food. Hygienic and sanitation practices applied in meat processing facilities are often insufficient to prevent contamination of processed meat products (Cox et al., 1989). *Listeria monocytogenes* is resistant to many food preservation methods and can increase to high numbers during refrigerated storage (Walker et al., 1990) and low oxygen tension (Lou and Yousef, 1999). Although the heat treatment (cooking) that ready-to-eat (**RTE**) meat and poultry products undergo eliminates the pathogen, recontamination may occur during postprocessing procedures such as peeling, slicing, and repackaging (Farber and Peterkin, 1999). Therefore, effective postprocessing antimicrobial interventions to inhibit growth of the pathogen are essential.

A novel approach to controlling *L. monocytogenes* in foods is the use of antimicrobial bacteriocins from lactic acid bacteria (Muriana, 1996). Nisin, a lanthionine-containing polypeptide produced by *Lactococcus lactis* ssp. *lactis* (Altena et al., 2000), is a bacteriocin with antimicrobial activity against *L. monocytogenes* (Bruno and Montville, 1993). The half-maximal lethal dose value was found to be similar to that of common salt (Montville and Bruno, 1994). Nisin is generally recognized as safe for use as a biopreservative in food systems. It is approved for use in meat and poultry products at 250 mg/kg in the finished product, 6.30 mg/kg in the finished product when used in casings, 5.0 mg/kg on cooked meat and poultry products, and 550 mg/kg of a blend of encapsulated nisin preparation (90.9%), rosemary extract (8.2%), and salt (0.9%) for frankfurt-

Received November 19, 2008.
Accepted October 21, 2009.
[1]Corresponding author: wsallyk@ufl.edu

ers and other similar cooked meat and poultry sausages (US Food and Drug Administration, 2008).

Applications that have been considered or investigated for nisin in poultry and meat systems include addition of nisin-producing *Lactococcus lactis* ssp. *lactis* to meat systems in an attempt to produce nisin in situ (Abee et al., 1994), antimicrobial dipping solutions (Zhang and Mustapha, 1999; Ariyapitipun et al., 2000; Samelis et al., 2005), nisin-coated casings (Luchansky and Call, 2004), direct addition of nisin into meat formulations (Gill and Holley, 2000; Samelis et al., 2002), antibotulinal agent for the partial replacement of nitrite in cooked meat systems (Abee et al., 1994), and use in canned meats as a means of reducing thermal processing time (Montville and Chen, 1998).

The mechanism of action for nisin is based on the disruption of the cytoplasmic cell membrane, as evidenced by the rapid efflux of small molecules from both whole cells and liposomes (Garcera et al., 1993; Abee et al., 1994; Winkowski et al., 1994). As a result, nisin depletes the proton motive force of sensitive cells and artificial liposomes (Gao et al., 1991; Bruno et al., 1992). Nisin acts through a multistep process, which includes binding of nisin to the cell, insertion into the membrane, and pore formation (Sahl, 1991; Garcera et al., 1993).

A major issue with the use of nisin in RTE poultry and meat products is determining the proper usage level and appropriate application method. This study proposes the addition of nisin treatment solutions to the RTE product during the final packaging step to ensure that the treatment solution is in direct contact with the ham and remains in the package throughout storage. The objectives of this study were to determine pH, anti-*Listeria* and general antimicrobial properties of nisin on RTE vacuum-packaged diced turkey ham inoculated with *L. monocytogenes*, and the usage level that would exert maximum antimicrobial effect during 63 d of storage.

MATERIALS AND METHODS

Inoculum Cultivation, Storage, and Preparation

Five reference strains of *L. monocytogenes*, 1/2a, 1/2b, 4b, Scott A, and 19115, were obtained from ABC Research Corporation in Gainesville, Florida, and used as the inoculum in this study. Initially, stock solutions were prepared by transferring each strain to test tubes containing 10 mL of tryptic soy broth (**TSB**, DF 0369-17-6, Difco Laboratories, Detroit, MI) using a flamed-sterilized 3-mm inoculation loop. The broth was incubated at 35°C for 24 h. After incubation, the cultures were poured into sterile 15-mL centrifuge tubes and centrifuged (Sorvall RC-5B, Dupont Instruments, Newton, CT; Type SS-34 rotor, Sorvall Instruments, Newton, CT) at 2,988 × *g* (Science Gateway, 2009) for 10 min at 16°C. The supernatant was discarded and the pellets were resuspended in 10 mL of sterile 0.1% buffered peptone water (**BPW**, DF O1897-17-4, Difco Laboratories) and recentrifuged. The supernatant was discarded and the pellets were resuspended in 1 mL of 3% TSB with 30% glycerol in a 2-mL cryovial (03-374-2, Corning Inc., Corning, NY), stored at −45°C, and used as the stock culture for the inoculation studies.

Twenty-four hours before conducting the study, 1 tube of each of the individual strains was removed from the freezer and allowed to thaw at room temperature for 10 min. A loopful of the cultures from each strain was transferred and mixed in test tubes containing 10 mL of 3% TSB, vortexed, and incubated at 35°C for 24 h. Two consecutive 24-h transfers of the stock cultures were conducted to obtain a culture in which the cells were in the same physiological state. After incubation, each culture was centrifuged at 2,988 × *g* for 10 min at 16°C, washed with sterile BPW, resuspended in BPW, mixed to form the 5-strain inoculum, and serially diluted with BPW to concentrations of 10^{-1} to 10^{-8}. Preliminary work was conducted to determine the concentration of inoculum needed to yield 4 to 5 log cfu/g on the ham samples.

Preparation of Nisin Solutions for Turkey Ham Samples

Nisin solutions containing 0.2, 0.3, 0.4, and 0.5% nisin were prepared using Nisaplin (a commercial product containing 10^6 IU of nisin/g, Danisco, Copenhagen, Denmark), 0.02 *N* food-grade HCl (7647-01-0, Fisher Scientific, Pittsburgh, PA), and salt (S9625-500G, Sigma Chemical, St. Louis, MO) based on the total formula weight. The HCl is used in meat systems primarily for ensuring that the nisin is in the solution before coming in contact with the complex meat system, which may interfere with the activity of nisin (Liu and Hansen, 1999; Rose et al., 1999).

Inoculation and Treatment

Commercially available turkey hams were purchased from a local supermarket as soon as the shipment arrived at the store and were used in this study. All hams purchased had sell-by dates of at least 60 d. The turkey hams were transported to the research laboratory on ice packs and stored in a walk-in cooler at 4 ± 1°C for no longer than 24 h before using. The hams were aseptically transferred from the vacuum-packaged bag to presterilized trays and were chopped into approximately 0.5-cm pieces as is typical for ham used in salads and sandwiches.

Approximately 3 kg of chopped turkey ham was placed on presterilized trays and was inoculated by spraying with a 10^8 cfu/mL *L. monocytogenes* inoculum. Inoculated samples were left to stand at room temperature for 20 min to allow for bacterial attachment to ensure a

final concentration of 10^4 cfu/g. Predetermined aliquots of the inoculated chopped turkey ham were aseptically weighed and placed into prelabeled vacuum bags (6.7 mL/m^2 for 24 h at 23°C and 0% RH; FoodSaver, T150-00011-002, Jarden Corporation, Rye, NY). Six turkey ham treatments were prepared containing either water only (negative control, no inoculum), *L. monocytogenes* inoculum plus water (positive control), or 0.2, 0.3, 0.4, or 0.5% nisin plus inoculum. The formulations consisted of diced turkey ham (90%) and water (10%). The actual amount of added water was adjusted based on the percentage of nisin desired in the formulation. The concentrated nisin treatments were added to each bag in predetermined aliquots to yield final concentrations of 0.2, 0.3, 0.4, and 0.5% nisin, respectively, based on total batch weight.

The inoculated chopped ham, water, and nisin solution was mixed manually in the vacuum bag to ensure proper distribution, sealed (FoodSaver, V2460, Jarden Corporation), and stored at 4 ± 1°C in a walk-in cooler for 63 d. Duplicate samples per treatment were analyzed after 0, 7, 14, 21, 28, 35, 42, 49, 56, and 63 d for aerobic plate count (**APC**), *L. monocytogenes*, lactic acid bacteria. and pH. Aerobic plate counts were performed on d 0 only to monitor sanitation and to ensure no cross contamination during sample preparation.

Microbiology and pH Analyses

Twenty-five grams of chopped turkey ham was transferred aseptically from the vacuum bag to a sterile stomacher bag (01-002-44, Fisher Scientific) containing 225 mL of sterile 0.1% BPW (DF O1897-17-4, BD Diagnostics, Sparks, MD) and was agitated for approximately 60 s. The appropriate serial dilutions were prepared by transferring 1.0 mL of the sample homogenate to 9 mL of sterile BPW. One milliliter of the dilutions was pipetted onto duplicate 3M Petrifilm for APC (6404, 3M, St. Paul, MN), and 1 μL was pipetted onto prepoured modified Oxford agar plates (DF0225-17-0, BD Diagnostics) with Oxford media supplement (DF0214-60-9, BD Diagnostics) for *L. monocytogenes* and all-purpose Tween agar (DF0654-17-0, BD Diagnostics) for lactic acid bacteria. The all-purpose Tween agar plates were placed into anaerobic jars (OXAN0020C, Fisher Scientific) with AnaeroGen 3.5-L packets (6535, Remel, Lenexa, KS) for generation of anaerobic conditions. All plates were incubated for 48 h at 35 ± 1°C. After incubation, colony-forming units from each plate were counted, recorded, averaged, and reported as colony-forming units per gram.

Immediately after the microbiological analyses were completed, pH values were recorded for each sample homogenate using an Accumet AB15 pH meter (Cole-Parmer, Vernon Hills, IL). The pH probe was placed into the sample homogenate and allowed to equilibrate for 1 min before the reading was taken. All pH readings were performed in duplicate.

Data Analysis

A complete randomized block design was employed. A total of 240 samples were analyzed (i.e., duplicate samples, 6 treatments, 10 storage days, 2 trials). The general GLM program (PROC GLM) of SAS (Version 8.02, SAS Institute, Cary, NC) was employed to determine differences between trials, among treatments and storage days, and treatment × day interaction. Any significant differences were determined using SAS Tukey multiple range test procedure at a level of significance of $\alpha = 0.05$.

RESULTS AND DISCUSSION

Aerobic Bacteria Analysis

In general, the APC revealed no evidence of poor sanitation or cross contamination (Table 1). Except for the positive control, all APC were less than 2.50 log cfu/g. The 0.4 and 0.5% nisin treatments resulted in lower ($P < 0.05$) APC when compared with the negative and positive controls. The 5 log cfu/g count for the positive control was indicative of the presence of the inoculum. The 0.4 and 0.5% nisin treatments resulted in 1.21 and 1.70 log reductions, respectively, when compared with the negative control and 3.97 and 4.40 log reductions, respectively, when compared with the positive control. The 0.2 and 0.3% nisin treatments resulted in less than 1 log reduction when compared with the negative control and 3.03 to 3.47 log reductions, respectively, when compared with the positive control.

pH Analysis

The pH values were similar ($P > 0.05$) for all treatments from d 0 to 49 (Table 2). On d 56, hams treated with 0.5% nisin had higher ($P < 0.05$) pH values when compared with all other treatments. On d 63, hams treated with 0.5% nisin had higher ($P < 0.05$) pH values than the negative and positive control hams. Except for hams treated with 0.3 and 0.5% nisin, the pH of all treatments decreased ($P < 0.05$) during storage. Although not always significant, the pH values decreased

Table 1. Mean aerobic plate counts for ready-to-eat turkey ham treated with various concentrations of nisin, inoculated with *Listeria monocytogenes*, and analyzed before storage[1]

Treatment	Day 0 (log cfu/g)
Negative control	2.28^{b}
Positive control	5.04^{a}
0.2% nisin	1.91^{bc}
0.3% nisin	1.57^{bcd}
0.4% nisin	1.07^{cd}
0.5% nisin	0.58^{d}

$^{a-d}$Means within a column lacking a common superscript differ ($P < 0.05$).

[1]Each mean value represents 8 individual measurements.

Table 2. pH measurements for ready-to-eat turkey ham treated with various concentrations of nisin, inoculated with *Listeria monocytogenes*, and stored at 4 ± 1°C for 63 d[1]

	Days of storage									
Treatment	0	7	14	21	28	35	42	49	56	63
Negative	$6.24^{a,x}$	$5.76^{a,y}$	$4.97^{a,z}$	$4.87^{a,z}$	$4.86^{a,z}$	$4.81^{a,z}$	$4.79^{a,z}$	$4.67^{a,z}$	$4.86^{b,z}$	$4.82^{b,z}$
Positive	$6.14^{a,x}$	$5.07^{a,y}$	$4.85^{a,y}$	$4.79^{a,y}$	$4.78^{a,y}$	$4.82^{a,y}$	$4.77^{a,y}$	$4.94^{a,y}$	$4.74^{b,y}$	$4.77^{b,y}$
0.2% nisin	$6.21^{a,x}$	$5.67^{a,xy}$	$5.46^{a,xy}$	$5.29^{a,xy}$	$5.19^{a,xy}$	$5.00^{a,y}$	$5.02^{a,y}$	$5.13^{a,xy}$	$5.01^{b,y}$	$4.86^{ab,y}$
0.3% nisin	$6.22^{a,x}$	$6.10^{a,x}$	$6.11^{a,x}$	$5.72^{a,x}$	$5.43^{a,x}$	$5.41^{a,x}$	$5.40^{a,x}$	$5.29^{a,x}$	$5.10^{b,x}$	$5.24^{ab,x}$
0.4% nisin	$6.26^{a,x}$	$5.85^{a,xy}$	$5.56^{a,xy}$	$5.41^{a,xy}$	$5.06^{a,y}$	$5.15^{a,y}$	$4.90^{a,y}$	$4.76^{a,y}$	$4.87^{b,y}$	$5.12^{ab,y}$
0.5% nisin	$6.21^{a,x}$	$6.14^{a,xy}$	$6.14^{a,xy}$	$6.08^{a,xy}$	$5.89^{a,xyz}$	$5.58^{a,yz}$	$5.79^{a,xyz}$	$5.67^{a,z}$	$5.80^{a,xyz}$	$5.83^{a,xyz}$

[a,b]Means within a column lacking a common superscript differ ($P < 0.05$).

[x–z]Means within a row lacking a common superscript differ ($P < 0.05$).

[1]Each mean value represents 8 individual measurements.

as the concentration of nisin decreased. This may be attributed to the production of numerous compounds such as acidic metabolites and carbonic acid that may decrease pH (Doyle et al., 2001).

L. monocytogenes Analysis

Listeria monocytogenes counts remained at 4.14 to 4.97 log cfu/g through 63 d of storage for the positive control, which confirmed that the desired *L. monocytogenes* inoculum concentration of 4 log cfu/g was achieved (Table 3). In general, all nisin treatments resulted in reduced ($P < 0.05$) *L. monocytogenes* counts when compared with the positive control on d 0, 7, and 49. No *L. monocytogenes* was isolated on the negative controls. On d 0, all nisin treatments resulted in 4 log reductions ($P < 0.05$) in *L. monocytogenes* when compared with the positive control. The 4 log reductions were also observed on d 7 for 0.4% nisin treatment and d 7 and 14 for the 0.5% nisin treatment ($P < 0.05$) when compared with the positive control. Two log reductions in *L. monocytogenes* were observed 21 through 63 d for samples treated with 0.5% nisin. The treatment × day interaction ($P < 0.05$) was attributed primarily to the increase ($P < 0.05$) in *L. monocytogenes* counts on d 42 through 63 for the 0.2% nisin treatment. Except for the 0.2% nisin treatment on d 42 through 63, no significant increase in *L. monocytogenes* counts occurred for hams treated with nisin through 63 d of storage. The 0.5% treatment demonstrated an extended lag phase through 63 d of storage, in which *L. monocytogenes* counts remained less than 1.96 log cfu/g. The reduction in *L. monocytogenes* counts on d 49 for all hams treated with nisin when compared with the positive control suggested that a continual antimicrobial effect was being exerted by the nisin treatments.

Lactic Acid Bacteria Analysis

Lactic acid bacteria populations increased as storage time increased for all treatments (Table 4). On d 0 and 49, lactic acid bacteria counts were significantly lower ($P < 0.05$) for all nisin treatments when compared with the positive control. Except for d 7, 14, and 21, hams treated with 0.5% nisin had lower ($P < 0.05$) lactic acid bacteria counts when compared with the positive and negative controls. Lactic acid bacteria counts were also lower ($P < 0.05$) for hams treated with 0.3% nisin ($P < 0.05$) when compared with positive and negative controls on d 56. This observation revealed the antimicrobial properties of nisin against lactic acid bacteria. It was discussed earlier that the pH values increased as the concentration of nisin increased to 0.5%. The data suggested that the shelf life of vacuum-packaged cured RTE poultry products may be extended with the use of 0.5% nisin by suppression of the growth of lactic acid bacteria.

In conclusion, this study revealed that nisin can be successfully incorporated into RTE turkey ham to control *L. monocytogenes* and lactic acid bacteria. The an-

Table 3. *Listeria monocytogenes* counts (log cfu/g) for ready-to-eat turkey ham treated with various concentrations of nisin, inoculated with *L. monocytogenes*, and stored at 4 ± 1°C for 63 d[1]

	Days of storage									
Treatment	0	7	14	21	28	35	42	49	56	63
Negative	$0.00^{b,y}$	$0.00^{c,y}$	$0.00^{b,y}$	$0.00^{b,y}$	$0.00^{b,y}$	$0.00^{b,y}$	$0.00^{b,y}$	$0.00^{e,y}$	$0.00^{b,y}$	$0.00^{c,y}$
Positive	$4.97^{a,y}$	$4.92^{a,y}$	$4.95^{a,y}$	$4.30^{a,y}$	$4.23^{a,y}$	$4.32^{a,y}$	$4.18^{a,y}$	$4.14^{a,y}$	$4.55^{a,y}$	$4.31^{a,y}$
0.2% nisin	$0.97^{b,z}$	$2.12^{b,yz}$	$3.22^{ab,yz}$	$2.45^{ab,yz}$	$2.94^{b,yz}$	$3.35^{a,yz}$	$3.57^{a,y}$	$2.97^{b,y}$	$3.61^{a,y}$	$3.52^{ab,y}$
0.3% nisin	$0.60^{b,y}$	$1.59^{bc,y}$	$1.96^{ab,y}$	$2.16^{ab,y}$	$2.95^{ab,y}$	$2.62^{ab,y}$	$2.57^{a,y}$	$1.83^{cd,y}$	$2.99^{ab,y}$	$3.10^{ab,y}$
0.4% nisin	$0.60^{b,y}$	$0.85^{bc,y}$	$2.29^{ab,y}$	$1.35^{ab,y}$	$2.50^{ab,y}$	$2.96^{ab,y}$	$3.06^{ab,y}$	$2.09^{c,y}$	$3.02^{ab,y}$	$2.82^{ab,y}$
0.5% nisin	$0.42^{b,y}$	$0.89^{bc,y}$	$0.17^{b,y}$	$1.66^{ab,y}$	$1.90^{ab,y}$	$1.95^{ab,y}$	$1.67^{ab,y}$	$1.01^{d,y}$	$1.89^{ab,y}$	$1.51^{bc,y}$

[a–e]Means within a column lacking a common superscript differ ($P < 0.05$).

[y,z]Means within a row lacking a common superscript differ ($P < 0.05$).

[1]Each mean value represents 8 individual measurements.

Table 4. Lactic acid bacteria counts (log cfu/g) for ready-to-eat turkey ham treated with various concentrations of nisin, inoculated with *Listeria monocytogenes*, and stored at 4 ± 1°C for 63 d[1]

	Days of storage									
Treatment	0	7	14	21	28	35	42	49	56	63
Negative	$3.25^{ab,x}$	$4.74^{a,wx}$	$5.66^{a,wx}$	$6.86^{a,w}$	$7.10^{a,w}$	$6.84^{a,w}$	$6.66^{a,w}$	$6.71^{a,w}$	$6.62^{a,w}$	$6.66^{a,w}$
Positive	$4.27^{a,x}$	$5.49^{a,wx}$	$5.90^{a,wx}$	$6.73^{a,w}$	$6.61^{a,w}$	$6.74^{a,w}$	$6.54^{a,w}$	$6.47^{a,w}$	$6.53^{a,w}$	$6.71^{a,w}$
0.2% nisin	$1.55^{b,y}$	$3.60^{a,x}$	$4.18^{a,wx}$	$4.65^{a,wx}$	$4.43^{ab,wx}$	$5.28^{ab,w}$	$5.43^{ab,w}$	$5.57^{b,w}$	$5.65^{ab,w}$	$5.12^{ab,wx}$
0.3% nisin	$1.99^{b,w}$	$2.44^{a,w}$	$2.79^{a,w}$	$3.46^{a,w}$	$3.94^{ab,w}$	$4.95^{b,w}$	$4.96^{b,w}$	$4.31^{c,w}$	$4.52^{bc,w}$	$4.94^{ab,w}$
0.4% nisin	$1.28^{b,z}$	$2.73^{a,yz}$	$3.43^{a,xy}$	$3.86^{a,wxy}$	$4.80^{ab,wxy}$	$5.41^{ab,wx}$	$5.18^{b,wx}$	$5.66^{b,w}$	$5.29^{abc,wx}$	$5.11^{ab,wx}$
0.5% nisin	$1.32^{b,wx}$	$1.02^{a,x}$	$1.59^{a,wx}$	$3.07^{a,wx}$	$3.13^{b,wx}$	$4.20^{b,w}$	$4.19^{b,w}$	$4.23^{c,w}$	$3.48^{c,wx}$	$3.00^{b,wx}$

[a–c]Means within a column lacking a common superscript differ ($P < 0.05$).

[w–z]Means within a row lacking a common superscript differ ($P < 0.05$).

[1]Each mean value represents 8 individual measurements.

timicrobial effectiveness of nisin increased as its concentration increased from 0.2 to 0.5% with the most effective antimicrobial level being 0.5%. The data revealed that 0.2, 0.3, and 0.4% nisin treatments resulted in lower ($P < 0.05$) *L. monocytogenes* when compared with the positive control initially (d 0) and for 1 wk (7 d) and on d 49. In comparison, the 0.5% nisin treatment resulted in lower ($P < 0.05$) *L. monocytogenes* counts initially (d 0) and for 2 wk (14 d) and on d 49 and 63. Although the counts on d 21, 28, 35, 42, and 56 were not significantly lower ($P > 0.05$) than the positive control, they were at least 2 log less than the positive control. The data for pH revealed that 0.5% nisin was effective in increasing pH of the ham and simultaneously decreasing lactic acid bacteria counts. This study also revealed that the 0.5% nisin treatment exerted maximum antimicrobial effects on *L. monocytogenes* and lactic acid bacteria during 63 d of storage. *Listeria monocytogenes* counts decreased from 4.97 log cfu/g on d 0 and remained less that 2 log cfu/g through 63 d of storage for the 0.5% nisin treatment.

REFERENCES

Abee, T., T. R. Klaenhammer, and L. Letellier. 1994. Kinetic studies of the action of lactacin F, a bacteriocin produced by *Lactobacillus johnsonii* that forms poration complexes in the cytoplasmic membrane. Appl. Environ. Microbiol. 60:1006–1013.

Altena, K., A. Guder, C. Cramer, and G. Bierbaum. 2000. Biosynthesis of the lantibiotic mersacidin: Organization of a type B lantibiotic gene cluster. Appl. Environ. Microbiol. 66:2565–2571.

Ariyapitipun, T., A. Mustapha, and A. D. Clarke. 2000. Survival of *Listeria monocytogenes* Scott A on vacuum packaged raw beef treated with polylactic acid, lactic acid, and nisin. J. Food Prot. 63:131–136.

Barbosa, W. B., L. Cabedo, H. J. Wederquist, J. N. Sofos, and G. R. Schmidt. 1994. Growth variations among species and strains of *Listeria monocytogenes*. J. Food Prot. 57:765–769.

Bell, C., and A. Kyriakides. 2005. Factors affecting the growth and survival of *Listeria monocytogenes*. Pages 62–69 in *Listeria*: A Practical Approach to the Organism and Its Control in Foods. 2nd ed. Blackwell Publishing Company, Ames, IA.

Bruno, M. E., A. Kaiser, and T. J. Montville. 1992. Depletion of the proton motive force by nisin in *Listeria monocytogenes* cells. Appl. Environ. Microbiol. 58:2255–2259.

Bruno, M. E. C., and T. J. Montville. 1993. Common mechanisms of bacteriocins from lactic acid bacteria. Appl. Environ. Microbiol. 59:3003–3010.

Cox, L., T. Kleiss, J. Cordier, C. Cordellana, P. Konkel, C. Pedrazzini, R. Beumer, and A. Siebenga. 1989. *Listeria* spp. in food processing, non-food and domestic environments. Food Microbiol. 6:49–61.

Doyle, M. P., L. R. Beuchat, and T. J. Montville. 2001. Page 383 in Food Microbiology: Fundamentals and Frontiers. 2nd ed. ASM Press, Washington, DC.

Farber, J. M., and P. I. Peterkin. 1991. *Listeria monocytogenes*, a food-borne pathogen. Microbiol. Rev. 55:476–511.

Farber, J. M., and P. I. Peterkin. 1999. Incidence and behavior of *Listeria monocytogenes* in meat products. Pages 505–564 in *Listeria*, Listeriosis and Food Safety. E. T. Ryser and E. H. Marth, ed. Marcel Dekker Inc., New York, NY.

Gao, F. H., T. Abee, and W. N. Konings. 1991. Mechanism of action of the peptide antibiotic nisin in liposomes and cytochrome *c* oxidase-containing proteoliposomes. Appl. Environ. Microbiol. 57:2164–2170.

Garcera, M. J., M. G. Elferink, A. J. Driessen, and W. N. Konings. 1993. In vitro pore-forming activity of lantibiotic nisin. Role of proton motive force and lipid composition. Eur. J. Biochem. 212:417–422.

Gill, A. O., and R. A. Holley. 2000. Inhibition of bacterial growth on ham and bologna by lysozyme, nisin and EDTA. Food Res. Int. 33:83–90.

Gombas, D. E., Y. Chen, R. S. Clavero, and V. N. Scott. 2003. Survey of *Listeria monocytogenes* in ready-to-eat foods. J. Food Prot. 66:559–569.

Liu, W., and J. N. Hansen. 1999. Some chemical and physical properties of nisin, a small-protein antibiotic produced by *Lactococcus lactis*. Appl. Environ. Microbiol. 56:2551–2558.

Lou, Y., and A. E. Yousef. 1999. Characteristics of *Listeria monocytogenes* important to food processors. Pages 131–224 in *Listeria*, Listeriosis and Food Safety. 2nd ed. Marcel Dekker Inc., New York, NY.

Luchansky, J. B., and J. Call. 2004. Evaluation of nisin-coated cellulose casings for the control of *Listeria monocytogenes* inoculated onto the surface of commercially prepared frankfurters. J. Food Prot. 67:1017–1021.

Montville, T. J., and M. E. Bruno. 1994. Evidence that dissipation of proton motive force is a common mechanism of action for bacteriocins and other antimicrobials proteins. Int. J. Food Microbiol. 24:53–74.

Montville, T. J., and Y. Chen. 1998. Mechanistic action of pelican and nisin: Recent progress and unresolved questions. Appl. Microbiol. Biotechnol. 50:511–519.

Muriana, P. 1996. Bacteriocins for control of *Listeria* spp in food. J. Food Prot. Suppl.:54–63.

Rose, N. L., P. Sporns, M. E. Stiles, and L. M. McMaullen. 1999. Inactivation of nisin by glutathione in fresh meat. J. Food Sci. 64:759–762.

Sahl, H. G. 1991. Pore formation in bacterial membranes by cationic lantibiotics. Pages 347–359 in Nisin and Novel Lantibiotics. J. Jung and H. G. Sahl, ed. Escom Publishers, Leiden, the Netherlands.

Samelis, J., G. K. Bedie, J. N. Sofos, K. E. Belk, J. A. Scanga, and G. C. Smith. 2002. Control of *Listeria monocytogenes* with

combined antimicrobials after post process contamination and extended storage of frankfurters at 4°C in vacuum packages. J. Food Prot. 65:299–307.

Samelis, J., G. K. Bedie, J. N. Sofos, K. E. Belk, J. A. Scanga, and G. C. Smith. 2005. Combinations of nisin with organic acids or salts to control *Listeria monocytogenes* on sliced pork bologna stored at 4°C in vacuum packages. Lebenson. Wiss. Technol. 38:21–28.

Science Gateway. 2009. Centrifuge rotor speed calculator. http://www.sciencegateway.org/tools/rotor.htm Accessed July 9, 2009.

US Food and Drug Administration. 2008. Nisin preparation: Affirmation of GRAS status as direct human food ingredient. Code of Federal Regulation 21CFR184.1538d. Office of the Federal Register, National Archives and Records Administration, Washington, DC.

Walker, S. J., P. Archer, and J. G. Banks. 1990. Growth of *Listeria monocytogenes* at refrigeration temperatures. J. Appl. Bacteriol. 68:157–162.

Winkowski, K., M. E. C. Bruno, and T. J. Montville. 1994. Correlation of bioenergetic parameters with cell death in *Listeria monocytogenes* cells exposed to nisin. Appl. Environ. Microbiol. 60:4186–4188.

Zhang, S., and A. Mustapha. 1999. Reduction of *Listeria monocytogenes* and *Escherichia coli* O157:H7 numbers on vacuum packaged fresh beef treated with nisin or nisin combined with EDTA. J. Food Prot. 62:1123–1127.